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N-Benzyl-linoleamide, a Constituent of *Lepidium meyenii* (Maca), Is an Orally Bioavailable Soluble Epoxide Hydrolase Inhibitor That Alleviates Inflammatory Pain

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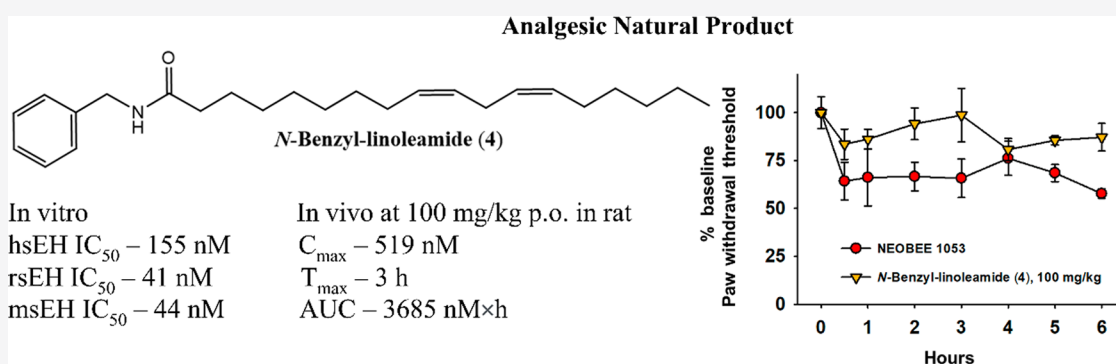Cite This: *J. Nat. Prod.* 2020, 83, 3689−3697

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Analgesic Natural Product

N-Benzyl-linoleamide (4)

In vitro
hsEH IC$_{50}$ – 155 nM
rsEH IC$_{50}$ – 41 nM
msEH IC$_{50}$ – 44 nM

In vivo at 100 mg/kg p.o. in rat
C$_{max}$ – 519 nM
T$_{max}$ – 3 h
AUC – 3685 nM×h

ABSTRACT: *Lepidium meyenii* (maca), a plant indigenous to the Peruvian Andes, recently has been utilized globally for claimed health or recreational benefits. The search for natural products that inhibit soluble epoxide hydrolase (sEH), with therapeutically relevant potencies and concentrations, led to the present study on bioactive amide secondary metabolites found in *L. meyenii*, the macamides. Based on known and suspected macamides, 19 possible macamides were synthesized and characterized. The majority of these amides displayed excellent inhibitory potency (IC$_{50}$ ≈ 20−300 nM) toward the recombinant mouse, rat, and human sEH. Quantitative analysis of commercial maca products revealed that certain products contain known macamides (**1−5, 8−12**) at therapeutically relevant total concentrations (≥3.29 mg/g of root), while the inhibitory potency of *L. meyenii* extracts directly correlates with the sum of concentration/IC$_{50}$ ratios of macamides present. Considering both its in vitro efficacy and high abundance in commercial products, *N*-benzyl-linoleamide (**4**) was identified as a particularly relevant macamide that can be utilized for in vivo studies. Following oral administration in the rat, compound **4** not only displayed acceptable pharmacokinetic characteristics but effectively reduced lipopolysaccharide-induced inflammatory pain. Inhibition of sEH by macamides provides a plausible biological mechanism of action to account for several beneficial effects previously observed with *L. meyenii* treatments.

E poxy fatty acids (EpFAs), derived from cytochrome P450 oxidation of polyunsaturated fatty acids, are lipid mediators with primarily anti-inflammatory, analgesic, antihypertensive, and antiapoptotic activities.[1−4] However, they are rapidly degraded to less bioactive dihydroxy fatty acids by soluble epoxide hydrolase (sEH). sEH inhibitors (sEHI) stabilize endogenous levels of EpFAs, enhancing their bioavailability and biological functions. Hence, inhibition of sEH is a novel and powerful therapeutic approach that could be utilized to tackle a number of unmet clinical needs. Thousands of highly potent urea-, amide-, and carbamate-based sEHI have been synthesized and characterized. Despite this, only a handful have entered clinical trials, and, currently, no drug on the market is used intentionally as an sEH inhibitor. One huge barrier is the extremely costly and time-

consuming approval process for synthetic drugs due to very stringent regulatory requirements.

Alternatively, sEHI from natural sources, such as botanicals, can act as possible nutraceuticals and, through dietary supplementation, might provide a significantly faster and inexpensive means to treat patients. This could be particularly important in developing countries. Previously, a number of chemicals from a variety of natural sources have been identified as sEHI,[5−12] including urea- and amide-based inhibitors from
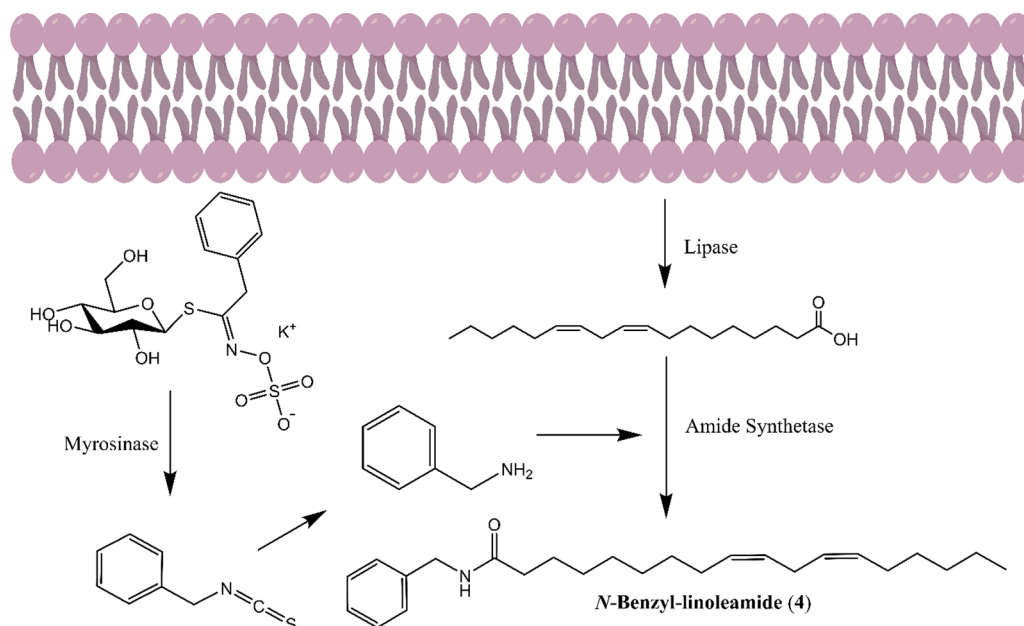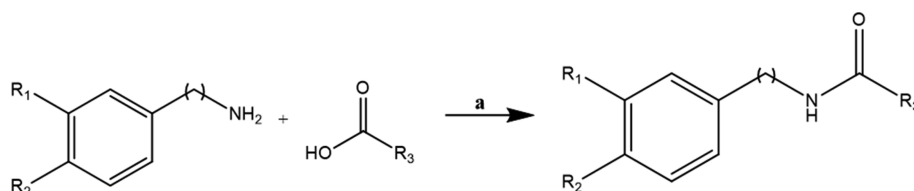
Figure 1. Schematic of hypothesized route for biosynthesis of macamides,[35−38] shown with the representative *N*-benzylamide of linoleic acid (**4**).

Scheme 1. General Synthesis of Compounds 2−19[a]

[a](a) EDC, DMAP (cat.), CH_2Cl_2.

plants in the order Brassicales.[13,14] However, a large number of these compounds have a low potency or their concentrations in the plant are insufficient for clinical application.

Lepidium meyenii Walp. (*L. meyenii*), commonly known as maca or "Peruvian ginseng", belongs to the family Brassicaceae and is indigenous to the high altitudes of the central Peruvian Andes. Its root is a native food crop, and there is rising global interest in *L. meyenii* products as herbal remedies.[15−17] Characterization of *L. meyenii* has revealed several secondary metabolites including glucosinolates, flavonolignans, and macamides.[18,19] Macamides are *N*-benzylamides of long-chain fatty acids (LCFAs) and are the key bioactive components of *L. meyenii*. They have demonstrated therapeutic potential against neurological disorders, including antidepressant effects[20] and protective action in models of Parkinson's disease.[21,22] Improvements in neural cell viability are attributed to the prevention of mitochondrial membrane depolarization and reduced induction of reactive oxygen species (ROS). Attenuation of oxidative stress has also been shown to mediate non-neurological effects including protection against erythrocyte hemolysis,[23] antifatigue effects in muscles,[24] and decreased lipid peroxidation in a diabetic rat model.[25] Moreover, combining a Chinese chive seed extract with a maca extract significantly synergized beneficial effects on male sexual health.[26]

Macamides have been characterized as inhibitors of fatty acid amide hydrolase (FAAH),[27,28] an enzyme that degrades neuroprotective endocannabinoids such as anandamide.[29]

Hence, FAAH inhibition has been proposed as a mechanism of action for macamides in the central nervous system. However, the potency is poor ($IC_{50} > 10\ \mu M$), insufficient for the degree of FAAH inhibition typically required to exert biological effects such as analgesia.[30] Thus, FAAH inhibition is unlikely to fully account for the neuroprotective activity of macamides. sEH inhibition stabilizes the mitochondrial dysfunction−ROS−endoplasmic reticulum stress axis and, consequently, underlies the amelioration of several pathologies.[3] Therefore, potentially it provides an alternative mode of action that captures macamide bioactivity. sEHI have also been shown to dramatically synergize with and expand the biological activity of FAAH inhibitors.[31]

Considering their structural similarities to previously identified amide pharmacophores containing sEHI,[32−34] 19 possible macamides were synthesized, characterized, and tested as novel inhibitors of sEH in vitro. Additionally, macamide content in a number of commercial maca products was quantified to investigate compound distribution and abundance among products. Finally, the most pertinent macamides were studied in vivo to assess pharmacokinetic parameters and analgesic effects in an inflammatory pain model.

■ RESULTS AND DISCUSSION

Macamide Design and Synthesis. A proposed route of biosynthesis of macamides involves LCFAs and benzylamine derivatives as substrates, which accumulate during traditional postharvest treatments as catabolic processes accelerate.[35−38]

LCFAs are released from membrane lipids while glucosinolates are broken down to benzyl isothiocyanates, converted to benzylamines, and enzymatically linked to the fatty acids, forming macamides (Figure 1). Thus, the macamides designed include a benzylamine moiety, which was either unsubstituted or possessed methoxy groups at the *meta/para* positions, similar to certain parent glucosinolates. They also contain a fatty acid portion, and a range of common saturated, mono- or polyunsaturated LCFAs were selected to account for effects of varying tail lengths and unsaturation sites.

Accordingly, the central synthetic method employed was amide synthesis (Scheme 1). In general, the appropriate LCFA was activated with 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide and a catalytic amount of 4-dimethylaminopyridine, under dry and inert conditions. This was followed by addition of benzylamine to generate compounds **2–7**, 3-methoxybenzylamine to generate compounds **8–14**, 3,4-dimethoxybenzylamine to generate compounds **15–17**, and phenylethylamine to generate compounds **18** and **19**.

Inhibition of Recombinant Human, Rat, and Mouse sEH. Inhibitory potency of the synthesized amides was tested against recombinant human, rat, and mouse sEH (Table 1). Compounds with polyunsaturated fatty acid tails were more potent than the corresponding benzylamide analogue with saturated tails (**4–7** vs **1 and 2** and **11–14** vs **8 and 9**). The difference was especially prominent relative to the saturated 18-carbon chains (**2 and 9**), which were between 2 and 3 orders of magnitude less potent and were the weakest of the characterized inhibitors. The addition of a single double bond at the C-9–C-10 position (**3 and 10**) increased potency by almost 2 orders of magnitude. These observations suggest olefinic bonds are important for the inhibitory potential of macamides with longer fatty acid tails. However, within inhibitor groups with two or more double bonds in their tails (**4–7** and **11–14**), there was little discernible difference in potencies. The addition of a *meta*-methoxy to the phenyl ring (**8–14**) generally decreased IC$_{50}$ values compared to unsubstituted analogues (**1–7**). This indicated that methoxy groups at *meta* positions can enhance sEH inhibition, as has been demonstrated with benzyl-containing urea-based natural sEH inhibitors.[14] A second, *para*-methoxy (**15–17**), however, did not improve inhibitory potency. In fact, it significantly reduced potency of compounds with a saturated 16-carbon tail (**15** vs **1 and 8**) and also lessened the efficacy of compounds with unsaturated tails (**16 and 17** vs **4 and 5**, and **11 and 12**). Insertion of a methylene between the phenyl ring and amide function (**18 and 19**) increased inhibitory potency toward the human enzyme by almost 1 order of magnitude, compared to analogues **4** and **5**. The influence might be due to reduced steric hindrance in the active site.

Macamide Levels in *L. meyenii* Products and Inhibitory Potency of Extracts. Thirteen commercially available *L. meyenii* root products were extracted and analyzed to quantify the amount of macamides present. Product types and sources are described in the Supporting Information (Table S1). Total macamide concentration estimates were based only on compounds with synthetic standards. Levels of macamides present varied significantly (>2 orders of magnitude) from product to product (Table 2), with an average of around 1.24 mg/g of root. The key determinant for differences is likely variability in postharvest treatment factors such as drying temperature, storage time, and air exposure, since these conditions dictate substrate release and subsequent

Table 1. Effects of Phenyl Substituents and Fatty Acid Chains on the sEH Inhibitory Potency of 19 Macamide-like Compounds

	R$_1$	R$_2$	R$_3$	Natural Product (+/-)	Human sEH IC$_{50}$ (nM)	Rat sEH IC$_{50}$ (nM)	Mouse sEH IC$_{50}$ (nM)	Human FAAH IC$_{50}$[a] (nM)
1	H	H		+	524	370	422	>100,000
2	H	H		+	>100,000	35,800	59,600	>100,000
3	H	H		+	893	198	265	16,700
4	H	H		+	155	41	44	10,800
5	H	H		+	134	23	28	41,800
6	H	H		-	123	40	50	N/A
7	H	H		-	111	29	43	N/A
8	OCH$_3$	H		+	235	113	159	N/A
9	OCH$_3$	H		+	16,077	28,676	89,850	N/A
10	OCH$_3$	H		+	241	84	126	11,000
11	OCH$_3$	H		+	92	43	38	10,300
12	OCH$_3$	H		+	76	9.3	27	13,700
13	OCH$_3$	H		-	55	22	73	N/A
14	OCH$_3$	H		-	63	18.6	49	N/A
15	OCH$_3$	OCH$_3$		+	7,059	15,910	>100,000	N/A
16	OCH$_3$	OCH$_3$		-	104	111	148	N/A
17	OCH$_3$	OCH$_3$		-	84	90	122	N/A

[a]Literature values[27]

	R$_1$	R$_2$	R$_3$	Natural Product (+/-)	Human sEH IC$_{50}$ (nM)	Rat sEH IC$_{50}$ (nM)	Mouse sEH IC$_{50}$ (nM)	Human FAAH IC$_{50}$[a] (nM)
18	H	H		-	28	33	91	N/A
19	H	H		-	15	15	39	N/A

[a]Literature values[27]

Table 2. Macamide Content and Inhibitory Potency of 13 *L. meyenii* Product Extracts

L. meyenii extract	total macamide concentrations (μg/g of root)	human sEH IC$_{50}$ (ng/mL)	mouse sEH IC$_{50}$ (ng/mL)
E1	22.4	11 700	12 600
E2	3602	329	138
E3	1804	377	169
E4	1780	450	139
E5	210	6380	6530
E6	217	4550	3400
E7	66.5	5,085	6290
E8	2480	422	207
E9	3,290	287	157
E10	301	2620	1701
E11	1490	980	456
E12	870	1606	869
E13	16.0	4380	>14 000

biosynthesis efficiency.[35−38] Certain extracts (e.g., E2 and E9) contained concentrations (≥3.29 mg/g of root) that were nearly 2 orders of magnitude greater than those of urea sEHI (<0.058 mg/g of root) found in plants such as *Pentadiplandra brazzeana* Baill.[13] This suggests that, under the right postharvest conditions, dried *L. meyenii* root extracts might have sufficiently high macamide concentrations to be of nutraceutical value as a source of naturally derived sEHI. To support this claim, the inhibitory potency of the *L. meyenii* extracts was measured for the human and mouse sEH (Table 2). Biological activity of extracts also varied dramatically, but the IC$_{50}$ values obtained inversely correlated with the amount of macamides in the extracts (Spearman's rank correlation coefficient $\rho_{\text{human sEH}} = -0.928$ and $\rho_{\text{mouse sEH}} = -0.956$), suggesting that the sEH inhibition was mostly due to the presence of macamides in the extracts.

Ten out of the 19 synthesized compounds were detected in all extracts, and these are known natural products,[26,27] specifically *N*-benzylamides of palmitic acid (**1**), stearic acid (**2**), oleic acid (**3**), linoleic acid (**4**), and α-linolenic acid (**5**) and their 3-methoxy-substituted analogues (**8−12**). Compound **15**, the 3,4-dimethoxy-substituted analogue of *N*-benzylpalmitamide (**1**), has also been detected previously in *L. meyenii*.[25] However, it was below the limit of quantitation (Table S2, Supporting Information) in the analyzed samples, possibly due to the difference in origin of maca. Amides with fatty acid tails containing 20 carbons, i.e., arachidonic acid (**6**, **13**) and eicosapentaenoic acid (**7**, **14**), were below the limit of quantitation (Table S2, Supporting Information), likely due to the relatively low concentrations of 20- and 22-carbon fatty acids in plant membrane lipids.[39,40] In the most abundant extract (E2), levels of compound **4** were the highest, with concentrations nearly three and four times greater than those of the next two most abundant macamides, **1** and **5**, respectively (Table 3). In order to gauge inhibitory contributions of individual macamides within the extract, the ratio of concentrations to the corresponding IC$_{50}$ values was determined (Table 3), to obtain a metric that accounted for both abundance in *L. meyenii* and potency toward sEH. The ratio was easily the greatest for macamide **4**, suggesting it is likely the most biologically relevant macamide found in *L. meyenii* roots to date. Macamide **5** was the next most relevant product, while the potential activity of the 3-methoxy-substituted analogues of **4** and **5** (i.e., **11** and **12**) as well as

Table 3. Concentrations and Abundance/IC$_{50}$ Ratios of Individual Macamides in Extract 2

compound	concentration (μg/g of root)	abundance/hsEH IC$_{50}$ (μg/nM)	abundance/msEH IC$_{50}$ (μg/nM)
1	680	1.30	1.61
2	269	<0.003	0.005
3	275	0.308	1.04
4	1640	10.6	37.3
5	464	3.46	16.6
8	39.6	0.168	0.249
9	24.0	0.001	<0.001
10	21.6	0.090	0.171
11	146	1.59	3.84
12	42.8	0.563	1.58

macamide **1** was also noteworthy. Higher abundance would largely account for the contribution of **1**, while, conversely, the excellent potency of **11** and **12** (Table 1) would compensate for their relatively lower levels in extracts.

Furthermore, the potency of extracts E1−13 directly correlated with the sum (Table S3, Supporting Information) of detected macamide abundance/IC$_{50}$ ratios (Spearman's rank correlation coefficient $\rho_{\text{human sEH}} = 0.929$ and $\rho_{\text{mouse sEH}} = 0.887$), signifying that the inhibitory potency toward sEH is likely a function of the concentrations and bioactivity of individual macamides present in the *L. meyenii* product.

Pharmacokinetics. Based on a combination of strong inhibitory potency and abundance in *L. meyenii* samples (Table 3), compounds **4** and **5** were selected for in vivo studies. To assess oral bioavailability of **4** and **5**, a pharmacokinetic study in rats was conducted to study their fate following oral administration of 100 mg/kg of each compound. A 96 h time-course of plasma concentrations of **4** and **5** was generated to assess key pharmacokinetic characteristics and is displayed in the Supporting Information (Figure S1).

Pharmacokinetic parameters (Table 4) indicated that the concentration of compound **4** peaked in the blood within 3 h

Table 4. Pharmacokinetic Parameters for a 100 mg/kg Oral Dose of Compounds 4 and 5[a]

compound	C_{max} (nM)	T_{max} (h)	AUC (nM × h)	$t_{1/2}$ (h)
4	519 ± 149	3	3690 ± 664	9
5	54 ± 11	6	723 ± 230	14.6

[a]Results are the mean ± SEM (n = 4/group).

and was around 1 order of magnitude greater than its rat sEH IC$_{50}$ (Table 1). However, the pharmacokinetic profile of compound **5** was poorer than that of **4**. It took double the time to reach its maximum blood concentration, which was nearly 1 order of magnitude smaller, relative to **4**. The total body exposure to the macamides (i.e., AUC) was also approximately five times lower for **5**, compared to **4**. It is possible the additional olefin bond in **5** increases susceptibility to secondary metabolism, autoxidation, and allylic and bisallylic hydroxylation,[41,42] decreasing in vivo stability.

Analgesia in an Inflammatory Pain Model. Compound **4** demonstrated antinociceptive effects in a lipopolysaccharide (LPS)-induced inflammatory pain model in rats. Baseline paw withdrawal thresholds were scored before administration of compounds and normalized to 100%. LPS results in lower than baseline scores, indicating a painful state. Oral administration
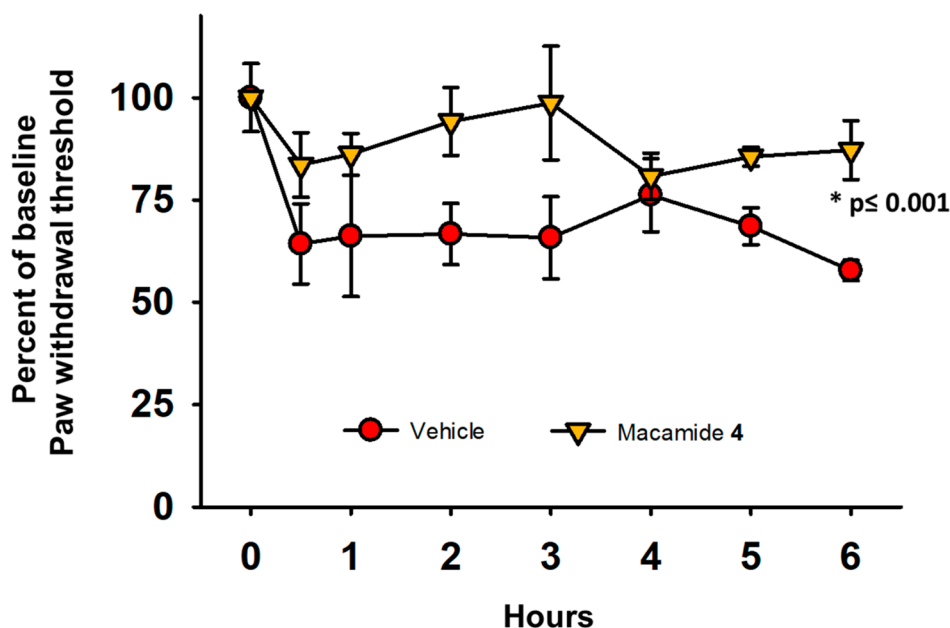
Figure 2. Macamide **4** (100 mg/kg) was efficacious against LPS-induced inflammatory pain in rat. Scores are the means ± SEM reported as percent of baseline (normalized to 100%), calculated as the score × 100/baseline score. The median value of efficacy was statistically significant compared to the vehicle control (NEOBEE 1053) over a 6 h time-course (Kruskal−Wallis one-way analysis of variance on ranks, $\alpha = 0.05$, *$p \leq 0.001$, $n = 4$/group).

of **4** (100 mg/kg) significantly increased paw withdrawal thresholds (interpreted as pain relief), relative to the vehicle control (Figure 2). Therapeutic effects persisted over a 6 h time-course, similar to activity of classic, synthetic sEHI against LPS-induced allodynia.[43] Effects of **5** were not significant (Figure S2, Supporting Information), probably due to its poor bioavailability.

The discovery of macamides as sEHI can at least partially explain the vast majority of biological effects observed with *L. meyenii* extract or macamide treatments. Through restoration of mitochondrial health and attenuation of oxidative stress, sEHI have proven therapeutically promising using in vitro and in vivo models of Parkinson's disease.[44−46] sEHI have also demonstrated antidepressant effects in mice, through improved brain-derived neurotrophic factor signaling in a stress model.[47] Mechanistically and/or functionally, the effects parallel macamide-mediated neuroprotection under these disease conditions.[20−22] Similarly, protection against ROS underlies the efficacy of sEHI against seemingly distinct pathologies[3] and mirrors macamide activity in dissimilar disease conditions tied together by oxidative stress.[23−25] The significantly greater potency toward sEH, compared to FAAH (Table 1), also strengthens the case for sEH inhibition as the more prevalent biological mechanism of action for macamides. However, macamides could in fact act as dual inhibitors of sEH and FAAH, potentially leading to synergism of biological effects, such as analgesia,[31] by facilitating both EpFA bioactivity and endocannabinoid-mediated agonism of cannabinoid (CB) receptors. The mixture of bioactive natural products in an *L. meyenii* extract could have further additive or synergistic effects and could provide another advantage over classic small-molecule therapeutics. Finally, macamides (such as compound **4**) themselves selectively bind to the CB1 receptor[48] and hence might directly exert some of the observed analgesic effects. The cannabimimetic activity could help explain the recreational use of maca, though it suggests perhaps some caution should be

exercised when considering *L. meyenii* for medicinal applications.

■ **CONCLUSION**

Nineteen potential macamides were synthesized, their inhibitory potency toward sEH was tested, and their levels in commercial *L. meyenii* products were quantified. These compounds, several of which are known natural products, were found to be a novel and promising class of potent sEHI. *N*-Benzyl-linoleamide (**4**) is the most therapeutically significant macamide studied so far, based on a combination of its dominant abundance in maca products, in vitro potency, and in vivo efficacy. Two unsaturation sites in the fatty acid tail appear to provide an optimal balance between inhibitory potency and oral bioavailability, leading to significant analgesia in an inflammatory pain model. The therapeutic relevance of total macamides in maca products depends on the postharvest treatment of *L. meyenii*, due to its significant influence on macamide biosynthesis. Certain samples contained levels that were nearly 2 orders of magnitude greater than those of sEHI found in previously identified dietary sources. Hence, careful selection and preparation of maca products is the key consideration in their potential application as nutraceuticals. In this study, analysis of macamides in *L. meyenii* products was conducted only using compounds with synthetic standards. However, it is probable other macamides exist and should be investigated in future studies. Current and new synthetic standards may facilitate efforts in breeding, postharvest handling, and other agronomic practices to increase the efficacy and concentration of macamides in commercial extracts.

■ **EXPERIMENTAL SECTION**

General Experimental Procedures. Compound **1** and most reagents required for synthesis are commercially available and were purchased from one of the following commercial vendors: Nu-Chek

Prep, Inc. (Elysian, MN), Sigma-Aldrich Chemical Co. (Milwaukee, WI), Fisher Scientific (Houston, TX), Eanmine LLC (Monmouth Jct, NJ), Oakwood Chemical (Estill, SC), Chem-Impex Inc. (Wood Dale, IL), or Combi-Blocks (San Diego, CA). All reactions were carried out in anhydrous solvents, under an atmosphere of nitrogen or argon and at room temperature. All chemicals purchased from commercial sources were used as received without further purification. NMR spectra were recorded on a 400 MHz Bruker Avance III HD Nanobay NMR spectrometer. Multiplicity is described by the abbreviations b = broad, s = singlet, d = doublet, t = triplet, m = multiplet. Chemical shifts (δ) are expressed as ppm. ^{1}H NMR spectra were referenced to the residual solvent peak at δ 7.26 (CDCl$_3$) or 2.51 (DMSO-d_6). ^{13}C NMR spectra were referenced to the solvent peak at δ 77.16 (CDCl$_3$) or 40.01 (DMSO-d_6). HRESIMS were recorded on a Thermo Electron LTQ-Orbitrap XL Hybrid mass spectrometer, equipped with an electrospray ionization (ESI) source operating in the positive-ion mode. Analytical TLC was performed on Merck TLC silica gel 60 F254 plates, and spots were revealed under 254 nm UV light or developed with a potassium permanganate stain. Flash chromatography was performed on silica gel (230−400 mesh) from Macherey Nagel. Commercial *L. meyenii* product extracts were analyzed via HPLC-MS/MS. An Agilent 1200 SL liquid chromatography series (Agilent Corporation, Palo Alto, CA, USA), utilizing a Kinetex C$_{18}$ 100 Å, LC 100 × 2.1 mm, 1.7 μm column, was employed. It was coupled to a 4000 Q-Trap tandem mass spectrometer (Applied Biosystems Instrument Corp.), equipped with an ESI source (Turbo V) operating in the positive-ion mode. Multiple reaction monitoring (MRM) transitions were optimized via direct infusion of standards.

Chemistry. General Synthetic Method for Compounds 2−19, as Shown with the Representative *N*-Benzyloctadeca-9Z,12Z-dienamide (4). Linoleic acid (2.00 g, 7.13 mmol), 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (1.71 g, 8.91 mmol), and 4-dimethylaminopyridine (catalytic amount) were stirred in dichloromethane (10 mL) for 15 min. Benzylamine (1.09 mL, 10.0 mmol) was added, and the mixture was stirred overnight. After completion (confirmed by TLC), the crude mixture was concentrated under reduced pressure, redissolved in hexanes, and purified by flash chromatography (ethyl acetate−hexanes 15:85 → 20:80). Yield: 2.21 g (84%).

N-Benzyloctadecanamide (2). Yield 29%; white powder, mp 89−91 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.38−7.29 (5H, m, aromatic), 5.70 (1H, bs, NH), 4.47 (2H, d, *J* = 5.6 Hz, benzylic), 2.23 (2H, t, *J* = 7.6 Hz, α-H), 1.72−1.64 (2H, m, β-H), 1.34−1.27 (28H, m, CH$_2$), 0.90 (3H, t, *J* = 6.8 Hz, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 173.00 (CC(=O)N), 138.46 (C), 128.71 (CH), 127.90 (CH), 127.84 (CH), 43.59 (CH$_2$), 36.84 (CH$_2$), 31.94 (CH$_2$), 29.72 (CH$_2$), 29.70 (CH$_2$), 29.67 (CH$_2$), 29.63 (CH$_2$), 29.52 (CH$_2$), 29.38 (CH$_2$), 29.37 (CH$_2$), 29.34 (CH$_2$), 25.80 (CH$_2$), 22.71 (CH$_2$), 14.14 (CH$_3$); MS/MS MRM transition (Q1 → Q3) *m/z* 374.30 [M + H]$^+$ → 91.00 [benzylium].

N-Benzyloctadec-9Z-enamide (3). Yield 90%; white powder, mp 48−50 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (1H, t, *J* = 6.0 Hz, NH), 7.33−7.21 (5H, m, aromatic), 5.37−5.29 (2H, m, vinylic), 4.25 (2H, d, *J* = 6.4 Hz, benzylic), 2.12 (2H, t, *J* = 7.6 Hz, α-H), 2.01−1.97 (4H, m, allylic), 1.53−1.50 (2H, m, β-H), 1.32−1.25 (20H, m, CH$_2$), 0.86 (3H, t, *J* = 7.2 Hz, CH$_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.54 (CC(=O)N), 140.26 (C), 130.11 (CH), 128.68 (CH), 127.61 (CH), 127.12 (CH), 42.41 (CH$_2$), 35.80 (CH$_2$), 31.75 (CH$_2$), 29.57 (CH$_2$), 29.30 (CH$_2$), 29.16 (CH$_2$), 29.13 (CH$_2$), 29.11 (CH$_2$), 29.06 (CH$_2$), 29.03 (CH$_2$), 27.08 (CH$_2$), 27.05 (CH$_2$), 25.78 (CH$_2$), 22.56 (CH$_2$), 14.42 (CH$_3$); MS/MS MRM transition (Q1 → Q3) *m/z* 372.30 [M + H]$^+$ → 91.10 [benzylium].

N-Benzyloctadeca-9Z,12Z-dienamide (4). Yield 84%; white powder, mp 30−32 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.38−7.30 (5H, m, aromatic), 5.74 (1H, bs, NH), 5.44−5.32 (4H, m, vinylic), 4.47 (2H, d, *J* = 6.0 Hz, benzylic), 2.80 (2H, t, *J* = 6.4 Hz, bisallylic), 2.23 (2H, t, *J* = 7.2 Hz, α-H), 2.10−2.04 (4H, m, allylic), 1.72−1.64 (2H, m, β-H), 1.40−1.33 (14H, m, CH$_2$), 0.92 (3H, t, *J* = 6.4 Hz, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.92 (CC(=O)N), 138.40 (C), 130.21 (CH), 130.03 (CH), 128.69 (CH), 128.02 (CH), 127.88

(CH), 127.81 (CH), 127.48 (CH), 43.96 (CH$_2$), 36.78 (CH$_2$), 31.90 (CH$_2$), 29.98 (CH$_2$), 29.33 (CH$_2$), 29.27 (CH$_2$), 29.24 (CH$_2$), 29.12 (CH$_2$), 27.18 (CH$_2$), 25.74 (CH$_2$), 25.60 (CH$_2$), 22.95 (CH$_2$), 14.07 (CH$_3$); HREIMS *m/z* 370.3117 [M + H]$^+$ (calcd for C$_{25}$H$_{40}$NO$^+$, 370.3104); MS/MS MRM transition (Q1 → Q3) *m/z* 370.30 [M + H]$^+$ → 91.00 [benzylium].

N-Benzyloctadeca-9Z,12Z,15Z-trienamide (5). Yield 83%; colorless oil; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.36−7.29 (5H, m, aromatic), 5.73 (1H, bs, NH), 5.45−5.31 (6H, m, vinylic), 4.46 (2H, d, *J* = 5.2 Hz, benzylic), 2.83 (4H, t, *J* = 6.4 Hz, bisallylic), 2.22 (2H, t, *J* = 7.6 Hz, α-H), 2.12−2.05 (4H, m, allylic), 1.72−1.68 (2H, m, β-H), 1.39−1.33 (8H, m, CH$_2$), 1.00 (3H, t, *J* = 7.2 Hz, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 173.20 (CC(=O)N), 138.61 (C), 131.96 (CH), 130.26 (CH), 128.80 (CH), 128.30 (CH), 128.26 (CH), 127.75 (CH), 127.70 (CH), 127.33 (CH), 127.14 (CH), 43.43 (CH$_2$), 36.65 (CH$_2$), 29.62 (CH$_2$), 29.33 (CH$_2$), 29.31 (CH$_2$), 29.19 (CH$_2$), 27.24 (CH$_2$), 25.82 (CH$_2$), 25.65 (CH$_2$), 25.56 (CH$_2$), 20.58 (CH$_2$), 14.32 (CH$_3$); HREIMS *m/z* 368.2958 [M + H]$^+$ (calcd for C$_{25}$H$_{38}$NO$^+$, 368.2948); MS/MS MRM transition (Q1 → Q3) *m/z* 368.30 [M + H]$^+$ → 108.10 [benzylaminium].

N-Benzylicosa-5Z,8Z,11Z,14Z-tetraenamide (6). Yield 91%; colorless oil; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.34−7.25 (5H, m, aromatic), 6.38 (1H, bs, NH), 5.45−5.33 (8H, m, vinylic), 4.39 (2H, d, *J* = 6.0 Hz, benzylic), 2.87−2.79 (6H, m, bisallylic), 2.23 (2H, t, *J* = 7.6 Hz, α-H), 2.14−2.05 (4H, m, allylic), 1.77−1.69 (2H, m, β-H), 1.42−1.29 (6H, m, CH$_2$), 0.92 (3H, t, *J* = 7.2 Hz, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.72 (CC(=O)N), 138.46 (C), 130.53 (CH), 129.13 (CH), 128.71 (CH), 128.22 (CH), 127.84 (CH), 127.52 (CH), 43.60 (CH$_2$), 36.06 (CH$_2$), 34.68 (CH$_2$), 31.61 (CH$_2$), 29.36 (CH$_2$), 29.09 (CH$_2$), 27.26 (CH$_2$), 26.73 (CH$_2$), 25.66 (CH$_2$), 22.66 (CH$_2$), 20.69 (CH$_2$), 14.12 (CH$_3$); MS/MS MRM transition (Q1 → Q3) *m/z* 394.30 [M + H]$^+$ → 91.00 [benzylium].

N-Benzylicosa-5Z,8Z,11Z,14Z,17Z-pentaenamide (7). Yield 90%; colorless oil; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.33−7.24 (5H, m, aromatic), 6.41 (1H, bs, NH), 5.43−5.30 (10H, m, vinylic), 4.39 (2H, d, *J* = 5.6 Hz, benzylic), 2.89−2.79 (8H, m, bisallylic), 2.21 (2H, t, *J* = 7.6 Hz, α-H), 2.13−2.06 (4H, m, allylic), 1.76−1.69 (2H, m, β-H), 0.99 (3H, t, *J* = 7.6 Hz, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.91 (CC(=O)N), 138.55 (C), 132.04 (CH), 129.19 (CH), 128.69 (CH), 128.61 (CH), 128.60 (CH), 128.28 (CH), 128.26 (CH), 128.15 (CH), 128.11 (CH), 127.88 (CH), 127.70 (CH), 127.36 (CH), 127.04 (CH), 43.46 (CH$_2$), 35.94 (CH$_2$), 26.74 (CH$_2$), 25.67 (CH$_2$), 25.60 (CH$_2$), 25.58 (CH$_2$), 20.60 (CH$_2$), 14.32 (CH$_3$); MS/MS MRM transition (Q1 → Q3) *m/z* 392.30 [M + H]$^+$ → 91.00 [benzylium].

N-(3-Methoxybenzyl)hexadecanamide (8). Yield 36%; white powder, mp 60−62 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.27−7.25 (1H, m, aromatic), 6.89 (1H, d, *J* = 7.6 Hz, aromatic), 6.84−6.83 (2H, m, aromatic), 5.75 (1H, bs, NH), 4.45 (2H, d, *J* = 5.6 Hz, benzylic), 3.82 (3H, s, H−C−O), 2.25 (2H, t, *J* = 7.2 Hz, α-H), 1.71−1.66 (2H, m, β-H), 1.34−1.28 (24H, m, CH$_2$), 0.92 (3H, t, *J* = 6.8 Hz, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.97 (CC(=O)N), 159.92 (CC(=C)O), 140.01 (C), 129.76 (CH), 120.04 (CH), 113.39 (CH), 112.99 (CH), 55.25 (O−CH$_3$), 43.57 (CH$_2$), 36.87 (CH$_2$), 31.94 (CH$_2$), 29.71 (CH$_2$), 29.67 (CH$_2$), 29.62 (CH$_2$), 29.51 (CH$_2$), 29.37 (CH$_2$), 29.34 (CH$_2$), 25.79 (CH$_2$), 22.70 (CH$_2$), 14.13 (CH$_3$); MS/MS MRM transition (Q1 → Q3) *m/z* 376.30 [M + H]$^+$ → 121.00 [3-methoxybenzylium].

N-(3-Methoxybenzyl)octadecanamide (9). Yield 83%; white powder, mp 77−79 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.22−7.18 (1H, m, aromatic), 6.83 (1H, d, *J* = 7.6 Hz, aromatic), 6.79−6.76 (2H, m, aromatic), 6.41 (1H, bs, NH), 4.34 (2H, d, *J* = 5.6 Hz, benzylic), 3.76 (3H, s, H−C−O), 2.18 (2H, t, *J* = 7.6 Hz, α-H), 1.66−1.58 (2H, m, β-H), 1.31−1.26 (28H, m, CH$_2$), 0.89 (3H, t, *J* = 7.2 Hz, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 173.25 (CC(=O)N), 159.82 (CC(=C)O), 140.22 (C), 129.98 (CH), 119.88 (CH), 113.25 (CH), 112.72 (CH), 55.10 (O−CH$_3$), 43.35 (CH$_2$), 36.66 (CH$_2$), 31.95 (CH$_2$), 29.74 (CH$_2$), 29.72 (CH$_2$), 29.71 (CH$_2$), 29.69 (CH$_2$), 29.67 (CH$_2$), 29.57 (CH$_2$), 29.43 (CH$_2$), 29.39 (CH$_2$), 25.86

(CH$_2$), 22.71 (CH$_2$), 14.13 (CH$_3$); MS/MS MRM transition (Q1 → Q3) m/z 404.30 [M + H]$^+$ → 121.00 [3-methoxybenzylium].

N-(3-Methoxybenzyl)octadec-9Z-enamide (10). Yield 98%; colorless oil; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.27−7.24 (1H, m, aromatic), 6.88 (1H, d, J = 7.2 Hz, aromatic), 6.84−6.82 (2H, m, aromatic), 5.76 (1H, bs, NH), 5.41−5.32 (2H, m, vinylic), 4.44 (2H, d, J = 5.6 Hz, benzylic), 3.82 (3H, s, H−C−O), 2.23 (2H, t, J = 7.2 Hz, α-H), 2.05−2.00 (4H, m, allylic), 1.69−1.64 (2H, m, β-H), 1.36−1.29 (20H, m, CH$_2$), 0.90 (3H, t, J = 6.8 Hz, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.95 (CC(=O)N), 159.91 (CC(=C)O), 140.03 (C), 130.01 (CH), 129.75 (CH), 120.02 (CH), 113.39 (CH), 112.95 (CH), 55.23 (O−CH$_3$), 43.55 (CH$_2$), 36.82 (CH$_2$), 31.92 (CH$_2$), 29.78 (CH$_2$), 29.72 (CH$_2$), 29.54 (CH$_2$), 29.33 (CH$_2$), 29.32 (CH$_2$), 29.28 (CH$_2$), 29.16 (CH$_2$), 27.24 (CH$_2$), 27.19 (CH$_2$), 25.78 (CH$_2$), 22.70 (CH$_2$), 14.13 (CH$_3$); MS/MS MRM transition (Q1 → Q3) m/z 402.30 [M + H]$^+$ → 121.00 [3-methoxybenzylium].

N-(3-Methoxybenzyl)octadeca-9Z,12Z-dienamide (11). Yield 39%; colorless oil; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.28−7.24 (1H, m, aromatic), 6.89 (1H, d, J = 7.6 Hz, aromatic), 6.84−6.82 (2H, m, aromatic), 5.75 (1H, bs, NH), 5.44−5.31 (4H, m, vinylic), 4.43 (2H, d, J = 5.6 Hz, benzylic), 3.82 (3H, s, H−C−O), 2.80 (2H, t, J = 6.8 Hz, bisallylic), 2.23 (2H, t, J = 7.6 Hz, α-H), 2.09−2.04 (4H, m, allylic), 1.69−1.66 (2H, m, β-H), 1.42−1.28 (14H, m, CH$_2$), 0.91 (3H, t, J = 6.8 Hz, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.91 (CC(=O)N), 159.91 (CC(=C)O), 140.01 (C), 130.24 (CH), 130.06 (CH), 129.76 (CH), 128.06 (CH), 127.91 (CH), 120.03 (CH), 113.41 (CH), 112.96 (CH), 55.24 (O−CH$_3$), 43.56 (CH$_2$), 36.82 (CH$_2$), 31.54 (CH$_2$), 29.72 (CH$_2$), 29.62 (CH$_2$), 29.36 (CH$_2$), 29.31 (CH$_2$), 29.27 (CH$_2$), 29.16 (CH$_2$), 27.21 (CH$_2$), 25.77 (CH$_2$), 25.64 (CH$_2$), 22.53 (CH$_2$), 14.09 (CH$_3$); MS/MS MRM transition (Q1 → Q3) m/z 400.30 [M + H]$^+$ → 121.00 [3-methoxybenzylium].

N-(3-Methoxybenzyl)octadeca-9Z,12Z,15Z-trienamide (12). Yield 71%; colorless oil; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.28−7.24 (1H, m, aromatic), 6.89 (1H, d, J = 7.2 Hz, aromatic), 6.84−6.82 (2H, m, aromatic), 5.77 (1H, bs, NH), 5.45−5.30 (6H, m, vinylic), 4.44 (2H, d, J = 5.6 Hz, benzylic), 3.82 (3H, s, H−C−O), 2.82 (4H, t, J = 6.0 Hz, bisallylic), 2.22 (2H, t, J = 7.6 Hz, α-H), 2.11−2.04 (4H, m, allylic), 1.69−1.66 (2H, m, β-H), 1.38−1.28 (8H, m, CH$_2$), 1.00 (3H, t, J = 7.6 Hz, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.95 (CC(=O)N), 159.91 (CC(=C)O), 140.02 (C), 131.98 (CH), 130.28 (CH), 129.75 (CH), 128.30 (CH), 128.27 (CH), 127.74 (CH), 127.13 (CH), 120.02 (CH), 113.40 (CH), 112.94 (CH), 55.24 (O−CH$_3$), 43.55 (CH$_2$), 36.81 (CH$_2$), 29.71 (CH$_2$), 29.59 (CH$_2$), 29.31(CH$_2$), 29.27 (CH$_2$), 29.15 (CH$_2$), 27.22 (CH$_2$), 25.77 (CH$_2$), 25.63 (CH$_2$), 25.54 (CH$_2$), 20.57 (CH$_2$), 14.29 (CH$_3$); MS/MS MRM transition (Q1 → Q3) m/z 398.30 [M + H]$^+$ → 121.00 [3-methoxybenzylium].

N-(3-Methoxybenzyl)icosa-5Z,8Z,11Z,14Z-tetraenamide (13). Yield 90%; colorless oil; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.28−7.24 (1H, m, aromatic), 6.88 (2H, m, aromatic), 6.84−6.83 (2H, m, aromatic), 5.73 (1H, bs, NH), 5.45−5.34 (8H, m, vinylic), 4.43 (2H, d, J = 6.0 Hz, benzylic), 3.82 (3H, s, H−C−O), 2.86−2.80 (6H, m, bisallylic), 2.24 (2H, t, J = 7.6 Hz, α-H), 2.17−2.04 (4H, m, allylic), 1.81−1.73 (2H, m, β-H), 1.45−1.28 (6H, m, CH$_2$), 0.90 (3H, t, J = 6.8 Hz, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.68 (CC(=O)N), 159.91 (CC(=C)O), 139.90 (C), 130.54 (CH), 129.78 (CH), 129.08 (CH), 128.83 (CH), 128.25 (CH), 128.16 (CH), 127.87 (CH), 127.53 (CH), 120.08 (CH), 120.04 (CH), 113.47 (CH), 112.96 (CH), 55.24 (O−CH$_3$), 43.60 (CH$_2$), 36.10 (CH$_2$), 31.52 (CH$_2$), 29.33 (CH$_2$), 27.23 (CH$_2$), 26.70 (CH$_2$), 25.65 (CH$_2$), 25.54 (CH$_2$), 22.58 (CH$_2$), 14.08 (CH$_3$); MS/MS MRM transition (Q1 → Q3) m/z 424.30 [M + H]$^+$ → 121.00 [3-methoxybenzylium].

N-(3-Methoxybenzyl)icosa-5Z,8Z,11Z,14Z,17Z-pentaenamide (14). Yield 97%; colorless oil; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.28−7.24 (1H, m, aromatic), 6.86 (1H, d, J = 7.2 Hz, aromatic), 6.83−6.81 (2H, m, aromatic), 5.78 (1H, bs, NH), 5.45−5.31 (10H, m, vinylic), 4.42 (2H, d, J = 5.6 Hz, benzylic), 3.81 (3H, s, H−C−O), 2.87−2.80 (8H, m, bisallylic), 2.24 (2H, t, J = 7.6 Hz, α-H), 2.17−2.05 (4H, m, allylic), 1.80−1.72 (2H, m, β-H), 0.99 (3H, t, J = 7.2 Hz, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.72 (CC(=O)N), 159.90 (CC(=

C)O), 139.90 (C), 132.07 (CH), 129.77 (CH), 129.10 (CH), 129.04 (CH), 128.80 (CH), 128.60 (CH), 128.29 (CH), 128.22 (CH), 128.10 (CH), 127.87 (CH), 127.01 (CH), 120.04 (CH), 113.47 (CH), 112.93 (CH), 55.24 (O−CH$_3$), 43.59 (CH$_2$), 36.09 (CH$_2$), 29.71 (CH$_2$), 26.70 (CH$_2$), 25.64 (CH$_2$), 25.55 (CH$_2$), 25.54 (CH$_2$), 20.57 (CH$_2$), 14.28 (CH$_3$); MS/MS MRM transition (Q1 → Q3) m/z 422.30 [M + H]$^+$ → 121.00 [3-methoxybenzylium].

N-(3,4-Dimethoxybenzyl)hexadecanamide (15). Yield 33%; white, amorphous solid; ^{1}H NMR (400 MHz, CDCl$_3$) δ 6.84 (3H, bs, aromatic), 5.66 (1H, bs, NH), 4.41 (2H, d, J = 6.0 Hz, benzylic), 3.89 (6H, s, H−C−O), 2.22 (2H, t, J = 7.6 Hz, α-H), 1.56−1.51 (2H, m, β-H), 1.34−1.31 (24H, m, CH$_2$), 0.86 (3H, t, J = 6.8 Hz, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.90 (CC(=O)N), 149.20 (CC(=C)O), 148.51 (CC(=C)O), 131.07 (C), 120.11 (CH), 111.23 (CH), 111.18 (CH), 55.96 (O−CH$_3$), 55.89 (O−CH$_3$), 43.45 (CH$_2$), 36.09 (CH$_2$), 31.65 (CH$_2$), 29.71 (CH$_2$), 29.69 (CH$_2$), 29.67 (CH$_2$), 29.63 (CH$_2$), 29.51 (CH$_2$), 29.37 (CH$_2$), 29.35 (CH$_2$), 29.07 (CH$_2$), 25.35 (CH$_2$), 22.66 (CH$_2$), 14.21 (CH$_3$); MS/MS MRM transition (Q1 → Q3) m/z 406.30 [M + H]$^+$ → 151.10 [3,4-dimethoxybenzylium].

N-(3,4-Dimethoxybenzyl)octadeca-9Z,12Z-dienamide (16). Yield 85%; white, amorphous solid; ^{1}H NMR (400 MHz, CDCl$_3$) δ 6.84 (3H, bs, aromatic), 5.68 (1H, bs, NH), 5.39−5.36 (4H, m, vinylic), 4.41 (2H, d, J = 6.0 Hz, benzylic), 3.89 (6H, s, H−C−O), 2.79 (2H, t, J = 6.4 Hz, bisallylic), 2.22 (2H, t, J = 7.8 Hz, α-H), 2.09−2.04 (4H, m, allylic), 1.79−1.72 (2H, m, β-H), 1.39−1.27 (14H, m, CH$_2$), 0.92 (3H, t, J = 6.4 Hz, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.84 (CC(=O)N), 149.20 (CC(=C)O), 148.51 (CC(=C)O), 131.08 (C), 130.23 (CH), 130.01 (CH), 128.07 (CH), 127.90 (CH), 120.10 (CH), 111.24 (CH), 111.18 (CH), 55.93 (O−CH$_3$), 55.87 (O−CH$_3$), 43.45 (CH$_2$), 36.86 (CH$_2$), 31.43 (CH$_2$), 29.62 (CH$_2$), 29.15 (CH$_2$), 29.07 (CH$_2$), 27.28 (CH$_2$), 27.21 (CH$_2$), 25.76 (CH$_2$), 25.28 (CH$_2$), 22.66 (CH$_2$), 14.10 (CH$_3$); HREIMS m/z 430.3324 [M + H]$^+$ (calcd for C$_{27}$H$_{44}$NO$_3^+$, 430.3316); MS/MS MRM transition (Q1 → Q3) m/z 430.30 [M + H]$^+$ → 151.10 [3,4-dimethoxybenzylium].

N-(3,4-Dimethoxybenzyl)octadeca-9Z,12Z,15Z-trienamide (17). Yield 87%; white, amorphous solid; ^{1}H NMR (400 MHz, CDCl$_3$) δ 6.84 (3H, bs, aromatic), 5.66 (1H, bs, NH), 5.42−5.33 (6H, m, vinylic), 4.41 (2H, d, J = 5.6 Hz, benzylic), 3.89 (6H, s, H−C−O), 2.83 (4H, t, J = 6.8 Hz, bisallylic), 2.22 (2H, t, J = 7.6 Hz, α-H), 2.12−2.05 (4H, m, allylic), 1.69−1.62 (2H, m, β-H), 1.40−1.32 (8H, m, CH$_2$), 1.00 (3H, t, J = 7.2 Hz, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.83 (CC(=O)N), 149.20 (CC(=C)O), 148.52 (CC(=C)O), 131.98 (C), 131.06 (CH), 130.24 (CH), 128.31 (CH), 128.25 (CH), 127.76 (CH), 127.12 (CH), 120.11 (CH), 111.25 (CH), 111.18 (CH), 55.95 (O−CH$_3$), 55.89 (O−CH$_3$), 43.36 (CH$_2$), 36.87 (CH$_2$), 31.60 (CH$_2$), 29.59 (CH$_2$), 29.27 (CH$_2$), 29.14 (CH$_2$), 29.07 (CH$_2$), 27.68 (CH$_2$), 25.78 (CH$_2$), 25.35 (CH$_2$), 22.66 (CH$_2$), 14.31 (CH$_3$); HREIMS m/z 428.3165 [M + H]$^+$ (calcd for C$_{27}$H$_{42}$NO$_3^+$, 428.3159); MS/MS MRM transition (Q1 → Q3) m/z 428.30 [M + H]$^+$ → 151.10 [3,4-dimethoxybenzylium].

N-Phenethyloctadeca-9Z,12Z-dienamide (18). Yield 82%; white powder, mp 31−33 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.36−7.21 (5H, m, aromatic), 5.71 (1H, bs, NH), 5.44−5.32 (4H, m, vinylic), 3.57−3.52 (2H, m, H−C−N), 2.86−2.78 (4H, m, bisallylic, benzylic), 2.15−2.04 (6H, m, allylic, α-H), 1.63−1.59 (2H, m, β-H), 1.41−1.28 (14H, m, CH$_2$), 0.91 (3H, t, J = 7.2 Hz, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 173.07 (CC(=O)N), 138.96 (C), 130.25 (CH), 130.07 (CH), 128.79 (CH), 128.65 (CH), 128.06 (CH), 127.92 (CH), 126.52 (CH), 40.48 (CH$_2$), 36.86 (CH$_2$), 35.74 (CH$_2$), 31.54 (CH$_2$), 29.63 (CH$_2$), 29.36 (CH$_2$), 29.27 (CH$_2$), 29.25 (CH$_2$), 29.15 (CH$_2$), 27.22 (CH$_2$), 25.74 (CH$_2$), 25.65 (CH$_2$), 22.59 (CH$_2$), 14.09 (CH$_3$); MS/MS MRM transition (Q1 → Q3) m/z 384.30 [M + H]$^+$ → 105.10 [1-phenylethan-1-ylium].

N-Phenethyloctadeca-9Z,12Z,15Z-trienamide (19). Yield 88%; colorless oil; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.35−7.20 (5H, m, aromatic), 5.73 (1H, bs, NH), 5.48−5.30 (6H, m, vinylic), 3.57−3.52 (2H, m, H−C−N), 2.85−2.81 (6H, m, bisallylic, benzylic), 2.15−2.05 (6H, m, allylic, α-H), 1.64−1.57 (2H, m, β-H), 1.39−1.28 (8H, m,

CH$_2$), 1.00 (3H, t, J = 7.8 Hz, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 173.09 (CC(=O)N), 138.97 (C), 131.98 (CH), 130.28 (CH), 128.78 (CH), 128.64 (CH), 128.30 (CH), 128.26 (CH), 127.74 (CH), 127.13 (CH), 126.51 (CH), 40.50 (CH$_2$), 36.84 (CH$_2$), 35.74 (CH$_2$), 29.72 (CH$_2$), 29.61 (CH$_2$), 29.25 (CH$_2$), 29.14 (CH$_2$), 27.22 (CH$_2$), 25.74 (CH$_2$), 25.63 (CH$_2$), 25.55 (CH$_2$), 20.57 (CH$_2$), 14.29 (CH$_3$); HREIMS m/z 382.3115 [M + H]$^+$ (calcd for C$_{26}$H$_{40}$NO$^+$, 382.3104); MS/MS MRM transition (Q1 → Q3) m/z 382.30 [M + H]$^+$ → 105.10 [1-phenylethan-1-ylium].

sEH Inhibition Assay. The assay was performed as previously described.[49] All IC$_{50}$ values for recombinant human, mouse, and rat sEHs were determined by a fluorescence-based assay system in a 96-well, serial dilution format. Nonfluorescent cyano(6-methoxy-naphthalen-2-yl)methyl *trans*-[(3-phenyloxiran-2-yl)methyl] carbonate (MNPC) was used as the assay substrate at a final concentration of 5 μM. MNPC is hydrolyzed by sEH to the fluorescent 6-methoxynaphthaldehyde. Formation of the product was measured by a Molecular Devices M-2 plate reader (λ_{ex} = 330 nm, λ_{em} = 465 nm). All measurements were performed in triplicate, and the means are reported. t-TUCB, a classic sEHI, was run in parallel, and the obtained IC$_{50}$ values were corroborated with reported literature values,[43] to validate the experimental results (Table S4, Supporting Information).

Extraction and Analysis of Macamides from *L. meyenii* Products. Products 1−7 (Table S1, Supporting Information) were first homogenized via mortar and pestle grinding. For products 1−12 (Table S1, Supporting Information), 1 g of *L. meyenii* root powder was extracted with a 40 mL mixture of hexanes−ethanol (7:1) at 50 °C, under ultrasonication for 15 min. Each suspension was filtered, evaporated under reduced pressure, and reconstituted in 5 mL of ethyl acetate. Aliquots were diluted in HPLC-grade methanol and filtered through a 0.22 μm filter prior to analysis. Product 13 (Table S1, Supporting Information) was directly diluted, filtered, and analyzed. Other solvent and extraction systems were also tested, and normalized extraction efficiencies are described in the Supporting Information (Table S5). Maca extract samples were placed in an autosampler, 10 μL aliquots were injected on the HPLC column, and macamide concentrations were quantified by HPLC-MS/MS analysis using standard curves and dilution factors. The HPLC trace for the mixture of macamides detected in the extract of product 2 is displayed in the Supporting Information (Figure S3).

Pharmacology. All animal experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) of University of California, Davis (approval nos. 21204 for Pharmacokinetics and 21509 for Inflammatory Pain Model to B.D.H.).

Pharmacokinetics. Male Sprague−Dawley (SD) rats (n = 4/group, 8 weeks old, 250−300 g), purchased from Charles River Laboratories, were used in the pharmacokinetic study of compounds 4 and 5. Each compound (100 mg/kg) was dissolved in 100% NEOBEE 1053 and administered via oral gavage. Whole blood (10 μL) was collected with a pipet from the tail vein, punctured by a lancet at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, and 96 h after dosing. Each blood sample was immediately transferred to a tube containing 100 μL of water with 0.1% EDTA, vortexed, and stored at −80 °C until analysis. According to a previously reported method,[50] the blood samples were processed, and compound concentrations were determined.

Inflammatory Pain Model. A von Frey assay measuring mechanical allodynia was performed, as previously described,[51] in male SD rats (n = 4/group). The study was conducted in a randomized and blinded manner. Mechanical withdrawal thresholds (MWT) were determined before dosing to establish a baseline score. Then, 1 mL of 100% NEOBEE 1053 vehicle, 100 mg/kg compound 4, or 100 mg/kg compound 5 was oral gavaged. Immediately following oral gavage, 50 μL of a 0.2 μg/mL solution of LPS in saline was injected intraplanarly in a hind (ipsilateral) paw. The rats were then assessed for MWT over a 6 h time-course. The ipsilateral MWT were measured three to five times per rat per time point, and scores are reported as the means of a group of rats.

■ ASSOCIATED CONTENT

ⓈⒾ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00938.

Descriptions of 13 commercial *L. meyenii* products, normalized extraction efficiencies, analytical method limits of quantitation, sums of abundance/IC$_{50}$ ratios, IC$_{50}$s of in vitro positive control, blood concentration profiles of compounds 4 and 5, analgesic efficacy of 5, HPLC trace of commercial *L. meyenii* product extract (E2), and ^{1}H and ^{13}C NMR spectra for new compounds (16, 17, and 19) (PDF)

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